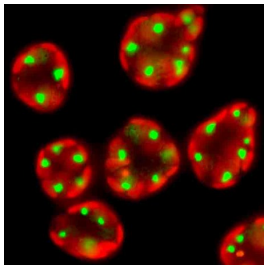
Apr 07, 2020 Version 2

Transformation of the chlorarachniophyte *Amorphochlora amoebiformis* by electroporation V.2



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Protocol status: Working
We use this protocol and it's working

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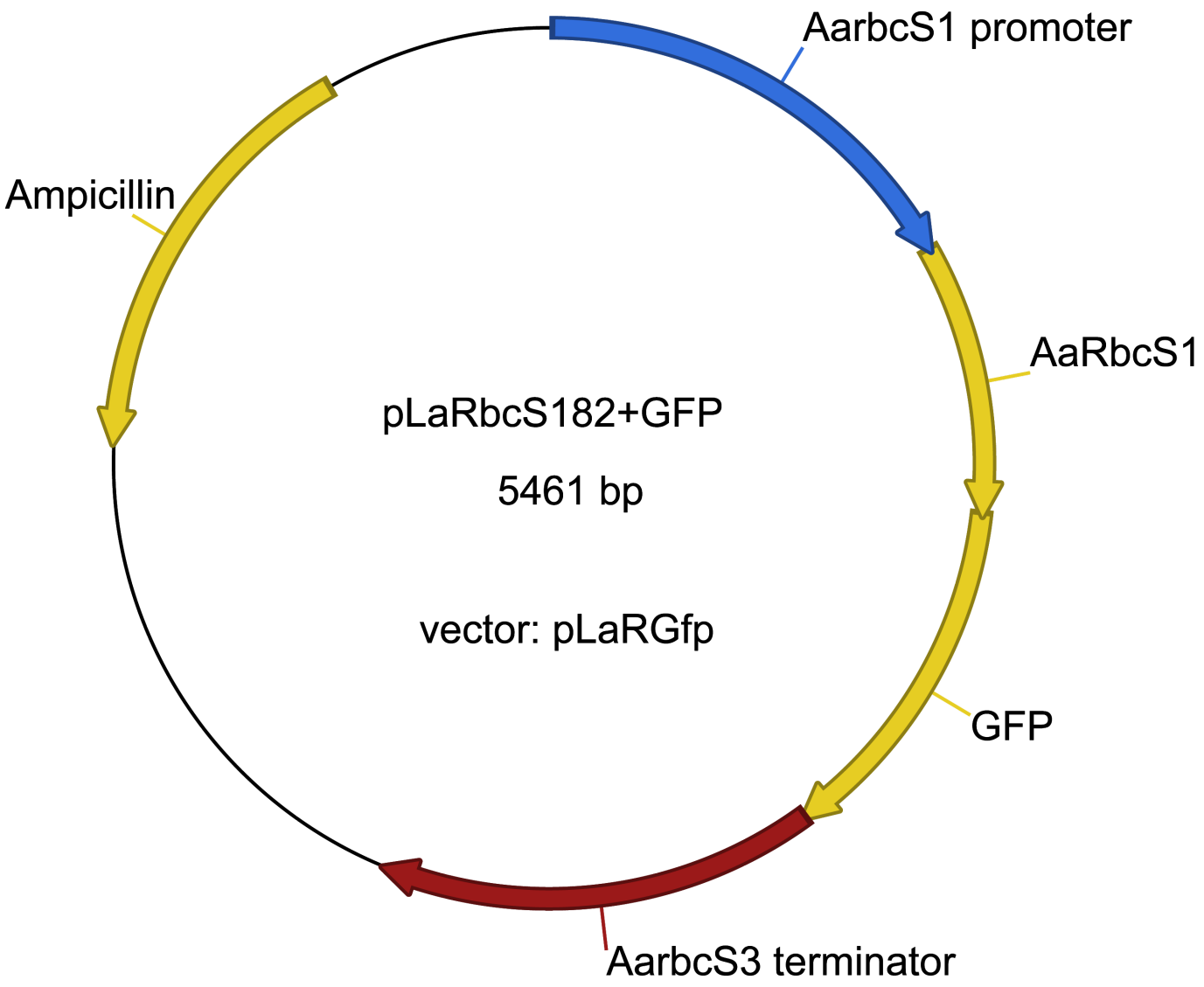
Materials

Plasmid information



>pLaRbcS182+GFP
GAACTCGAGCAGCTGCACACATTAACATTCATGTGGATAATAAATATAATATATATATATATATATATTATATATATATATATATATACAA

Hirakawa Y. Ishida K. (2010) Internal plastid-targeting signal found in a RubisCO small subunit protein of a chlorarachniophyte alga. The Plant Journal. 64: 402-410.

Plasmid preparation

1 Propagate plasmid DNA (pLaRGfp or its derivatives, such as pLaR182+GFP) in the *Escherichia coli* strain DH5α.

CITATION

Hirakawa, Y., Ishida, K. (2010). Internal plastid-targeting signal found in a RubisCO small subunit protein of a chlorarachniophyte alga. The Plant Journal.
LINK
10.1111/j.1365-313X.2010.04334.x

CITATION

Hirakawa, Y., Kofuji, R., Ishida, K. (2008). Transient transformation of a chlorarachniophyte alga, Lotharella amoebiformis (chlorarachniophyceae), with uidA and egfp reporter genes. Journal of Phycology.
LINK
10.1111/j.1529-8817.2008.00513.x

2 Purify plasmid DNA from 200 mL culture of *E. coli* by a Qiagen Plasmid Maxi Kit (Qiagen).

3 Adjust plasmid DNA concentration to 3-5 µg/µL with distilled water.

Cell culture

4 Culture *Amorphochlora amoebiformis* (CCMP2058) cells in 500 mL Erlenmeyer flasks containing 200 mL ESM medium at 20°C under white illumination (50-80 µmol photons·m^{-2}·s^{-1}) on a 14:10 hours light:dark cycle for a week.

5 After decanting medium, resuspend cells adhered to the bottom of flasks by gentile pipetting with 2 to 3 mL ESM medium (use a glass pipette). Approximately 4×10^7cells can be obtained from a flask.

Electroporation

6 Harvest a total of 5×10^6 cells by centrifugation at 2,000 g for 5 sec (use a mini centrifuge).

7 Resuspend cell pellet in 100 µL of Gene Pulser electroporation buffer (Bio-Rad) with 10 µL of plasmid DNA at the room temperature.

8 Transfer cell solution into electroporation cuvette with 0.2 cm gap (Bio-Rad).

9 Electroporate cells with a 25 ms square wave pulse at 120 V using Gene Pulser Xcell Electroporation System.

10 Add 0.9 mL fresh ESM medium to cuvette immediately after electroporation.

11 Transfer cells to glass bottom well plate/petri dish, and add an appropriate volume of ESM medium.

12 Incubate cells for 24 hours before observation of GFP fluorescence.

Citations

Step 1

Hirakawa, Y., Kofuji, R., Ishida, K.. Transient transformation of a chlorarachniophyte alga, Lotharella amoebiformis (chlorarachniophyceae), with uidA and egfp reporter genes
10.1111/j.1529-8817.2008.00513.x

Step 1

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